A novel, rapid method for a noncultured autologous skin cell suspension that preserves cell viability and wound healing capacity

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Directed by Professor Sung Phil Chung

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When I stopped my job in Yonsei University in 2002, I wondered if I can get doctorate in the future. 8 years later from that time, my boss Dr. Kim Kyungsik, the CEO of Medical Group Bestian, recommended my doctors' degree. I didn't consider my doctor's degree at all until Dr. Kim's recommendation. So I appreciate him greatly.

I wrote my thesis for doctorate in last spring. My thesis supervisor, professor Kim Seungho, gave me several advises for completion before unexpected death. I was in deep sorrow from his death because I respected my professor from my internship in Yonsei medical center and I was very proud of myself to be a disciple of him. I pray for the repose of his soul thorough this acknowledgement.

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Finally I thank to my wife, Anna Kim. She always give me material and spiritual support. She also had some trouble due to her master's degree. However, she always smiled for me. I'd like to share my pleasure to get doctor's degree with my wife.

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ABSTRACT

A novel, rapid method for a noncultured autologous skin cell suspension that preserves cell viability and wound healing capacity

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Objective: Noncultured autologous keratinocyte suspensions have been used for wound healing in burned patients. However, this process is time-consuming and potentially harmful due to the use of chemical agents. Here, we aimed to prepare noncultured autologous keratinocyte suspensions using a tissue homogenizer instead of chemical agents.

Methods: Cell number and viability were measured using trypan blue exclusion assays of homogenized skin tissues from burn patients. Cell types were identified by analysis of cell markers using reverse transcription polymerase chain reaction and by scanning electron microscopy (SEM). Immunofluorescence assays were performed to distinguish cell types in suspension. Wound healing capacity was analyzed by measuring growth factor expression by enzyme-linked immunosorbent assay. The potential clinical applicability of noncultured skin cell suspensions was analyzed using a nude rat model.

Results: After optimization of the homogenizer settings, cell viabilities ranged from 52% to 89%. Keratinocytes, fibroblasts, and melanocytes were identified in the suspension, and SEM images showed evidence of keratinocyte-like cell morphology. Several growth factors, including epidermal growth factor and basic fibroblast growth factor, were present in the cell suspensions. The rat model showed that these cell suspensions accelerated epithelialization and skin adnexa regeneration.

Conclusions: Noncultured autologous keratinocyte suspensions could be generated using a tissue homogenizer without suppressing cell viability in vitro or wound healing potential in vivo. Additionally, this process was rapid and did not require the use of chemical agents

Key words : keratinocyte, non-cultured skin cell suspension, burn

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I. INTRODUCTION

Epidermal replacement is the most important step in the management of burn patients to restore the skin barrier. Skin grafting is widely used for burn patients and is considered the gold standard treatment for skin replacement. However, some limitations still exist in the management of patients with extreme burns because of insufficient areas of nonburned skin when using autologous grafts. Many methods, such as meshed autografting,[1] combinations of autografting and allografting,[2] and autografting with cultured keratinocytes,[3] have been used to solve this problem. Among these methods, cultured epithelial autografts (CEAs) have been shown to be useful when donor sites are sparse.[4] However, CEAs have several disadvantages, including the requirement for long (3–4-week) culture periods to prepare appropriate cell sheets.[5] Such long culture periods may increase the risk of sepsis, which is the major cause of death in patients with extreme burns. Additional challenges of CEAs include poor clinical uptake and low cost-effectiveness.[6,7] Thus, novel methods for rapid, efficient generation of appropriate materials for skin grafting are needed.

Recently, a noncultured skin cell suspension (NCSS) called ReCell was introduced into clinical practice. ReCell allows immediate processing of small split-thickness biopsy specimens with trypsin to produce an autologous cell

suspension containing keratinocytes, melanocytes, fibroblasts, and Langerhans cells.[8] This cell suspension can be applied at a ratio of 1:80 to the burn wound without delays to culture cells. Thus, because no culture is required and the process is quick, this cell suspension could be used for on-site application in the operation room. However, ReCell is still somewhat time-consuming because preparation of trypsin is required for separating the epidermis from the dermal layer. Additionally, approval for the use of this medical device is needed from national organizations for health and welfare; this process of approval may take many years in some countries due to the use of chemicals.

In this study, we aimed to generate an NCSS rapidly and without the use of chemicals. We hypothesized that the use of tissue homogenization would yield a skin cell suspension with sufficient numbers of skin cells and growth factors to promote healing of damaged skin.

II. MATERIALS AND METHODS

1. Tissue harvesting and preparation of the NCSS

This study was approved by the Institutional Review Board of our hospital. We harvested small pieces of skin from burn patients undergoing a split thickness skin graft (STSG) procedure. Informed consent was obtained from all patients before collection or use of skin materials. After the STSG, the collected skin was immediately soaked with phosphate-buffered saline (PBS) and washed with 70% ethanol to remove contamination. The tissue was then washed two times with PBS and minced into small pieces using a razor blade or scissors. The tissues pieces were then placed into a small tube containing ceramic beads with 1–2 mL PBS. A tissue homogenizer (Bertin Technology, France) was used for cell detachment by homogenization twice at 5000 rpm for 30 s each. After homogenization, a cell strainer was used to isolate the final product as a liquid cell suspension with a volume of approximately 1–2 mL.

2. Optimal condition setting

To determine the optimal homogenization process for maintaining the viability of cells and integrity of subcellular components, we compared various tissue thicknesses ($8/1000^{th}$ versus $10/1000^{th}$ of an inch), bead materials (ceramic versus metal), and bead sizes (14 versus 28 mm).

3. Cell viability and counting

Prepared cell suspensions were maintained in PBS and then centrifuged at 250 × *g* for 5 min. NCSSs were resuspended in medium with trypan blue, and the total cell number and number of viable cells were determined using a hemocytometer. The number of cells was counted in a specific number of squares, and the dilution factor was used to calculate the original concentration in cells/mL.

4. Scanning electron microscopy (SEM)

The cells were fixed with 1.5% glutaraldehyde buffered in 0.1 M sodium cacodylate (Sigma, St. Lois, MO, USA) after 24-h incubation to allow the viewing of individual cells. The cells were then postfixed in 1% osmium tetroxide (Sigma) followed by 1% tannic acid (Sigma) as a mordant. Samples were dehydrated through a series of alcohols followed by further dehydration. The final dehydration was carried out in hexamethyldisilazane (Sigma), followed by air-drying. The samples were sputter coated with gold before examination with an HR-SEM (JSM-7000F/1V, JEOL, Japan).

5. Isolation of total RNA and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated using Easy-blue reagent (Intron, Korea) according to the manufacturer's instructions. RNA was reverse transcribed using M-MLV reverse transcriptase (Intron). Single-stranded cDNA was amplified by PCR with primers for keratin 10, involucrin, vimentin, tyrosinase related protein-1 (TRP-1), tyrosinase related protein-2 (TRP-2), microphthalmia-associated transcription factor (MITF), and β-actin (as an internal control for normalization). The primer sequences for these targets have been described in previous studies and are as follows: keratin 10, 5′-GAGCCTCGTGACTACAGCAA-3′ forward and 5′-GTTCTTCTTGAGATAGGCCA-3′ reverse; keratin 19, 5′-TTTGAGACGGAACAGGCTCT-3′ forward and 5′-TCTTCCAAGGCAGCTTTCAT-3′ reverse; involucrin, 5′-AAGGGAGAAGTATTGCTTCC-3′ forward and 5′-TGGGGAAGTAAGGATTCCTATAGTAAC-3′ reverse; TRP-1, 5′-ATCTGCACGGATGACTTGAT-3′ forward and 5′-GGCGTGTCAAATAAACCAAC-3′ reverse; TRP-2, 5′-GAGGTGCGAGCCGACACAAG-3′ forward and 5′-

CGGTGCCAGGTAACAAATGC-3′ reverse; MITF, 5′-GTGCAGACCCACCTGGAAAAC-3′ forward and 5′-AGTTAAGAGTGAGCATAGCCATAG-3′ reverse; vimentin, 5′-GAGAACTTTGCCGTTGAAGC-3′ forward and 5′-TCCAGCAGCTTCCTGTAGGT-3′ reverse; and β-actin, 5′-GCGAGAAGATGACCCAGATCATGTT-3′ forward and 5′-GCTTCTCCTTAATGTCACGCACGAT-3′ reverse.

6. Western blotting

Protein from the NCSS prepared from the harvested skin tissue was collected by lysing cells in RIPA buffer (ELPIS, Korea). Quantitative analysis of protein concentrations was carried out using Bicinchoninic acid (BCA) assays. Equal amounts of protein were then separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with 10% gels. The proteins were then transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). Membranes were blocked with 5% skim milk in phosphate-buffered saline with Tween-20 (PBS-T) and incubated at 4°C overnight with anti-pan cytokeratin (sc-15367, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-vimentin (sc-32322, Santa Cruz Biotechnology), and anti-β-actin antibodies (sc-47778, Santa Cruz Biotechnology). After washing three times with PBS-T, the membranes were incubated with secondary antibodies (sc-2004 or sc-2005, as appropriate; Santa Cruz Biotechnology) for 1 h.

7. Immunocytochemical staining

Immunocytochemical staining was performed to evaluate the expression of pan-cytokeratin), vimentin, and HMB-45 using appropriate antibodies (sc-15367, sc-32322, and sc-59305, respectively; Santa Cruz Biotechnology). Cells within the NCSS were fixed in 4% buffered neutral formalin solution permeabilized in Triton X-100 solution for 10 min at 25°C and then washed

three times with PBS. Cells were then incubated for 60 min with 1:50–1:200 dilutions of anti-pan-cytokeratin, anti-vimentin, or anti-HMB-45 antibodies. After washing the samples three times with PBS for 1 min, samples were incubated with fluorescently tagged secondary antibodies (sc-2004 or sc-2005, as appropriate; Santa Cruz Biotechnology). Nuclei were stained with DAPI (sc-3598, Santa Cruz Biotechnology). The images were obtained by confocal fluorescence microscopy (LSM5, Zeiss, USA)

8. Growth factor analysis

The concentrations of human epidermal growth factor (hEGF), basic fibroblast growth factor (bFGF), and β-defensin2 in NCSSs were measured by enzyme-linked immunosorbent assay (ELISA) using kits purchased from Komabiotech (K0331115, K0331192, and K0331208, respectively; Komabiotech, Korea). These growth factors were tested in cell-conditioned solution (1×10^6 cells/mL in PBS) according to the manufacturer's instructions. Briefly, 96-well plates were precoated with specific antibodies targeting the appropriate growth factors. Standards or test samples were added to the wells, and growth factor-specific antibodies were added and then washed with buffer. A streptavidin-peroxidase conjugate was then added, and unbound conjugates were removed by washing. TMB was catalyzed by streptavidin-peroxidase to produce a blue color product that changed to yellow after adding acidic stop buffer. The intensity of yellow color was directly proportional to the amount of growth factor captured on the plate and was measured using a VersaMax Microplate Reader (Molecular Devices, USA). The concentrations of hEGF, bFGF, and β-defensin2 were expressed as pg/mL.

9. Analysis of the wound healing capacity of NCSSs in rats

Randomly selected male nude rats weighing 90–130 g (5 weeks of age) were used to evaluate the wound healing capacity of NCSSs. All animal studies were

approved by the Ethics Committee of our University. Nude rats were anesthetized with 0.15 mL of a mixture of Virbac Zoletil 50 (Virbac, France) plus Rompun 2% (Bayer, Germany) injected into the right femoral area. The hair was shaved from the back of each rat, and a full skin thickness excisional wound (2 × 2 cm) extending through the panniculosus carnosus was created within the shaved area. Rats were randomly divided into the NCSS and control groups (n = 3 each). In the NCSS group, each wound was treated with 1 mL of NCSS, which was prepared from a portion of skin tissue measuring 1 × 1 cm. Each rat was treated with NCSSs prepared from a different patient. In the control groups, PBS alone was applied to the wound. To protect the wounds from infection and contamination, we used a multilayered dressing comprised of Bactigras (Smith & Nephew, Canada); meshed Vaseline gauze containing chlorohexidine, which was placed directly on the wound and did not stick to the wound; mupirocin ointment (Newgenpharm, Korea); several sheets of gauze, which were fixed with Renofix (TNL, Korea); and a CPK bandage (Farmaban, Spain) to avoid wound detachment. The dressing was changed every day. Nude rats were sacrificed by cervical dislocation after 1 or 2 weeks (n = 3 rats per treatment/control at each time). Wound biopsies were fixed in 4% buffered neutral formalin solution, and tissue blocks were sectioned at 10 μm thickness. Deparaffinized sections were stained with hematoxylin and eosin (Mayer's, USA). The evaluation of skin regeneration was determined by macroscopic and histopathological examinations by a pathologist.

10. Statistical analysis

Data were analyzed using SPSS ver 18.0. Mann-Whitney U tests were carried out to compare the mean scores between groups. Differences with *p* values of less than 0.05 were considered significant

III. RESULTS

1. Optimization of conditions

First, we determined the optimal settings for homogenization of the tissue. We found that optimal tissue homogenization was achieved when the tissue was homogenized twice at 5000 rpm for 30–45 s (data not shown). Under the above conditions, we also found that more cells were obtained and higher viability was observed when tissues that were $8/1000^{th}$ of an inch thick were used (Table 1).

Table 1. Comparison of various conditions for NCSSs under constant homogenization conditions

	Condition	Viable cells ($\times 10^6$ cells/cm^2)	Cell viability (%)
Tissue thickness (inch)	8/1000	5–10	70–85
	10/1000	10–20	60–70
Bead materials	Ceramic	5–10	65–90
	Metal	2–5	50–70
Bead size	14 mm	3–7	60–75
	28 mm	5–10	70–90

Additionally, ceramic beads yielded more viable cells than metal beads, and 28-mm beads yielded more viable cells than 14-mm beads. NCSSs were prepared from the skin from eight patients using these parameters, and cell viabilities ranged from 52% to 89% (Table 2).

Table 2. Cell counts and viability per cm^2 of NCSS for each sample

Patient no.	Sex/age (years)	Total cells/cm^2	Viable cells/cm^2	Cell viability (%)
1	M/70	2.4×10^6	1.24×10^6	52
2	F/37	1.07×10^7	7.68×10^6	72
3	M/36	8.96×10^6	5.12×10^6	57
4	F/69	2.32×10^6	1.68×10^6	72
5	F/47	1.29×10^7	1.15×10^7	89
6	M/62	2.35×10^7	1.49×10^7	64
7	M/40	7.04×10^6	4.52×10^6	64
8	F/36	1.14×10^7	1.15×10^7	80
Mean ± SD		$9.90 \times 10^6 \pm 6.75$	$7.26 \times 10^6 \pm 4.95$	69 ± 12

2. Cell identification

Cells exhibited round shapes similar to the morphology of keratinocytes, as shown using trypan blue assays of NCSSs. Scanning electron microscopy of NCSSs revealed keratinocyte-like cells showing extracellular matrix (Figure 1).

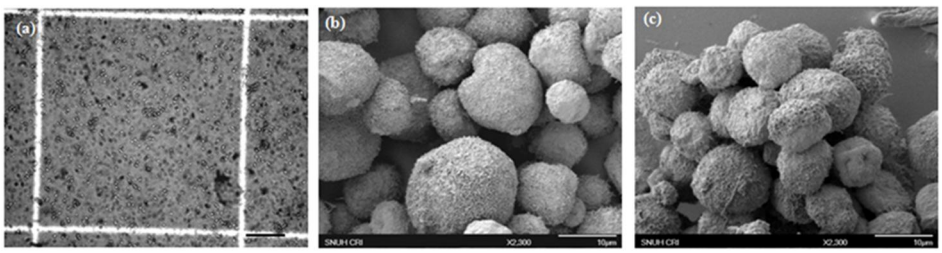


Fig. 1. Morphology of isolated noncultured skin cell suspensions (NCSSs)
The morphologies and sizes of cells within NCSSs were analyzed using trypan blue assays (a) and SEM imaging (b,c). The image in b shows a normal keratinocyte, while the image in c shows cells from the NCSS. Magnifications: 200× (a) and 2300× (bc,). The scale bars in the SEM images are 10 μm

3. Gene expression from cells within the NCSSs

Next, we analyzed the identities of the cells within the NCSSs by evaluating cell markers using RT-PCR. Keratin 10, keratin 19, and involucrin were used to identify keratinocytes; TRP1, TRP2, and MITF were used to identify kelanocytes; and vimentin was used to identify fibroblasts. All cell markers were expressed except TRP1 (Figure 2a), supporting that NCSSs were composed of keratinocytes, melanocytes, and fibroblasts. Western blotting showed marked expression of pan-cytokeratin (Figure 2b).

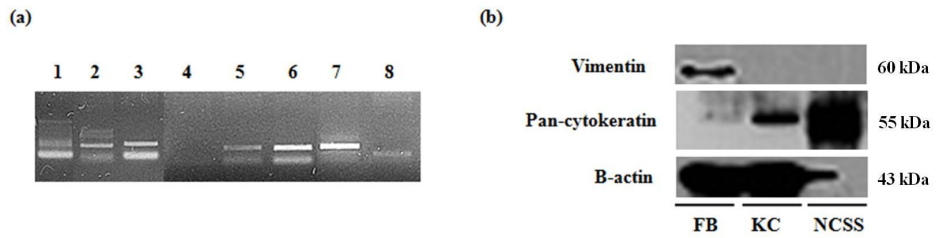


Fig. 2. Expression of skin cell markers in cells from NCSSs
Keratinocyte, melanocyte, and fibroblast markers were evaluated in cells within NCSSs using RT-PCR (a) and western blotting (b). Lane 1: keratin 10, lane 2: keratin 19, lane 3: involucrin, lane 4: TRP1, lane 5: TRP2, lane 6: MITF, lane 7: vimentin, lane 8: β-actin.

4. Immunostaining of NCSSs

Immunocytochemistry was used to further analyze the characteristics of NCSSs. Pan-cytokeratin staining showed the presence of keratinocytes, while vimentin staining showed the presence of fibroblasts (Figure 3). Additionally, HMB-45 was weakly expressed in melanocytes (data not shown). These data indicated that NCSSs consisted of abundant keratinocytes and some fibroblasts and melanocytes.

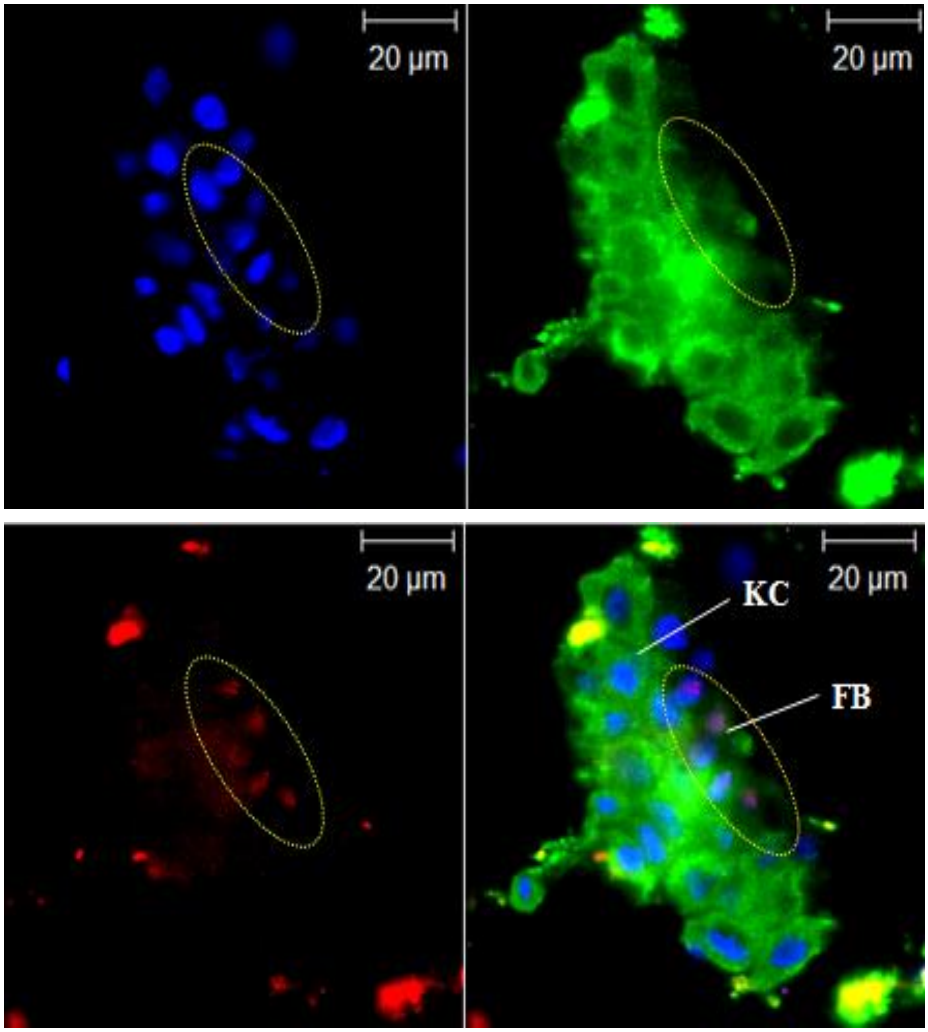


Fig. 3. Characterization of NCSSs by immunostaining

Immunocytochemistry (ICC) was performed for detection of pan-cytokeratin and vimentin. The scale bar indicates 20 μm. Fluorescence images are representative of cells form 10 sequential fields of view in two independent experiments. Blue: DAPI, nuclei; green: FITC, pan-cytokeratin; red: Cy5, vimentin

5. Growth factors expressed by cells within the NCSSs

To investigate the presence of growth factors and cytokines that may affect wound healing, we performed ELISAs of NCSSs. We observed a 2–4-fold increase in the expression of EGF in NCSSs compared with control (PBS), and a 2–3-fold increase in bFGF in NCSSs compared with PBS. The levels of β-defensin2 were increased by 2–18-fold in NCSSs compared with PBS (Figure 4).

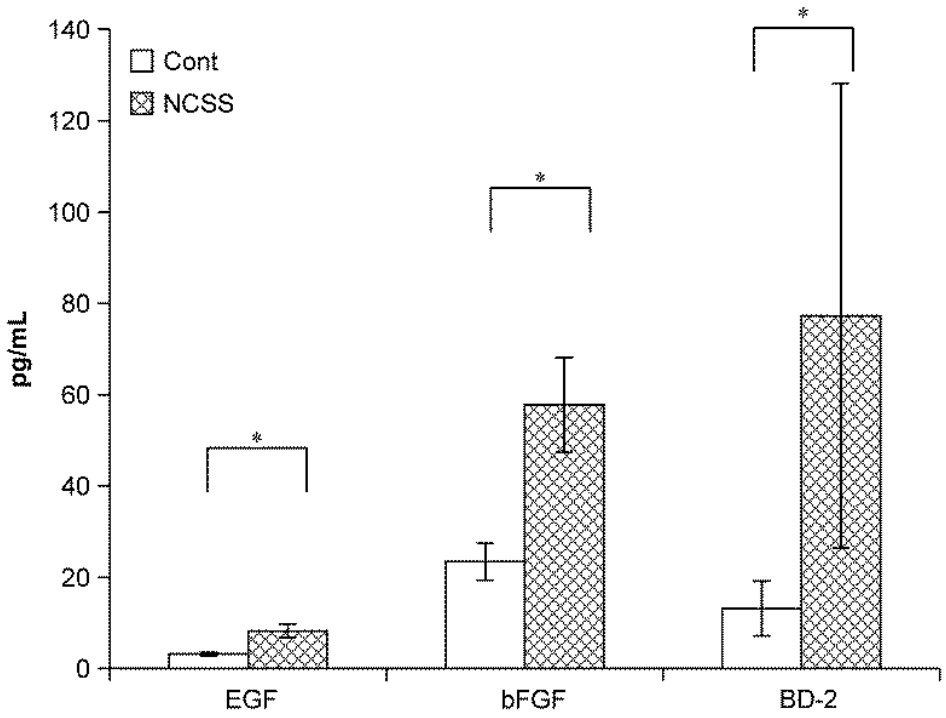


Fig. 4. Quantitative data analysis of cytokines in NCSSs
The expression of growth factors in the NCSS was assessed by enzyme-linked immunosorbent assay (ELISA). Data are shown as the mean ± standard deviation for each treatment group and are representative of three independent experiments (*, $p < 0.05$).

6. Wound healing in an in vivo model

Finally, we compared the rate and quality of wound healing in rats treated with NCSSs or PBS (control). On day 7 after wounding, wound healing was increased in rats treated with NCSSs compared with control rats treated with PBS only. Skin adnexa regeneration and collagen maturation were markedly different on days 7 and 14 (Figure 5). Moreover, rats in the NCSS group showed marked mature collagen replacement by Masson's trichome stain (Figure 6).

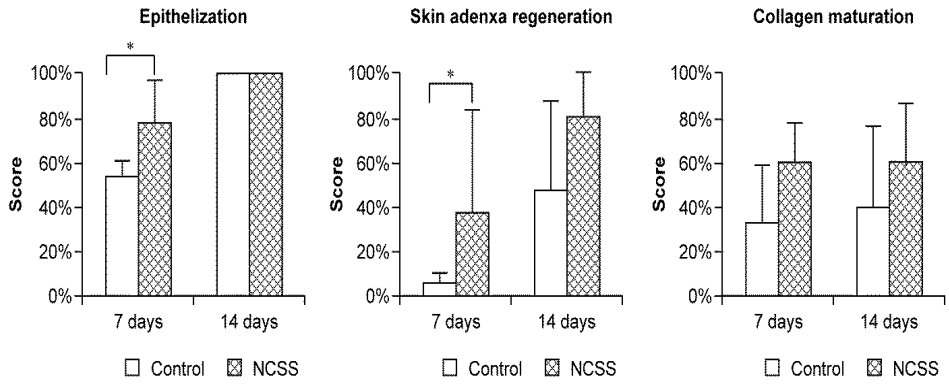
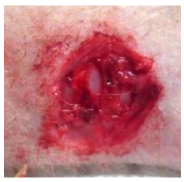
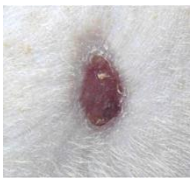
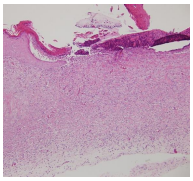
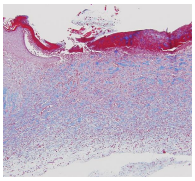
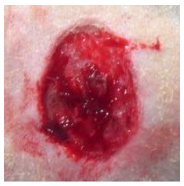
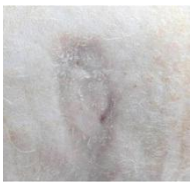
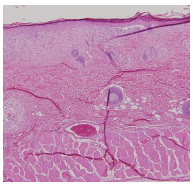
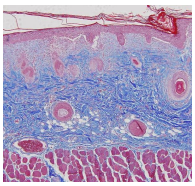


Fig. 5. Effects of the NCSS on wound healing in a nude rat model

Epithelialization, skin adnexa regeneration, and collagen maturation were analyzed at 7 and 14 days after application of the NCSS or control treatment. Data are shown as the mean ± standard deviation for each treatment group (*, $p < 0.05$).

	0 Day	7 Day		
		Macro	H&E	M&T
CONT				
NCSS				

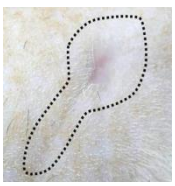
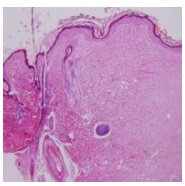
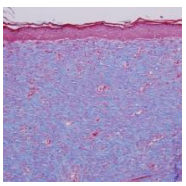
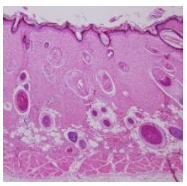
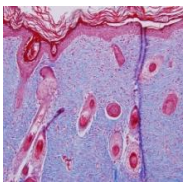
	14 Day		
	Macro	H&E	M&T
CONT			
NCSS			

Fig. 6. Macroscopic and microscopic findings of wound healing after application of the NCSS

Hematoxylin and eosin staining was performed to analyze skin adnexa generation. Collagen maturation was evaluated using Masson's trichrome staining. Macro-scale bars = 1 cm, micro-scale bars = 100 μm

IV. DISCUSSION

In this study, we developed a method for preparation of NCSSs from skin tissues of burn patients without the use of chemical agents. Our analyses showed that the NCSSs were composed of keratinocytes, melanocytes, and fibroblasts and substantially improved wound healing compared with PBS alone in a rat model.

Early wound closure is known to reduce mortality in patients with extreme burns. Because of the necessity to perform skin grafts rapidly in such patients, CEAs and other cultured cells have limited application in skin grafting. Therefore, the use of noncultured skin cells, which are applied to the wound immediately after harvesting skin from the donor site, would be highly advantageous in these patients. The first use of noncultured keratinocyte suspensions for treating burn wounds and chronic leg ulcers was reported by Hunyadi et al. in 1988.[9] Moreover, in a porcine model, Navarro et al. reported that noncultured keratinocyte suspensions accelerated wound healing, improved the quality of epithelialization, and restored melanocyte populations compared to control treatment.[10] The product ReCell, which was introduced in 2005, was also shown to provide improved aesthetic and functional outcomes [8]. Another system, called CellSpray XP, was introduced in 2008 and used a noncultured autologous keratinocyte suspension to promote burn wound closure.[11] While these systems represented a major improvement in the time to grafting (2 days for CellSpray XP and only 20–30 min with ReCell), our system allowed for application of NCSSs within only 10 min, further shortening the operation time. Moreover, the ReCell system requires the use of trypsin, which is known to degrade cell-cell and cell-matrix adhesions and cleave cell surface proteins; this may lead to dysregulation of cell functions. Indeed, Huang et al.[12] have shown that trypsinization causes downregulation of proteins related to growth and metabolism and upregulation of proteins related to apoptosis. These findings imply that trypsin used for cell subculture may have adverse effects on cell physiology. Indeed, some investigators have suggested that the poor outcomes

of CEA application may be associated with loss of integrin, an adhesive receptor, which is usually destroyed by trypsin.[13] Thus, our method, which does not use trypsin or other chemical components, may avoid these issues.

In this study, our preparation procedure involved the use of tissues measuring 8/1000th of an inch, which were disrupted using 28-mm ceramic beads. From the preparation on ice, we achieved yields of 1×10^6 viable cells/cm^2 within 10 min (cell viability: 69%). Thus, our method was comparable to the use of ReCell, which yielded 1.7×10^6 viable cells/cm^2 (cell viability: ~70%).[14] However, our method was 2–3 times faster than ReCell, supporting its potential superiority. Additionally, SEM imaging, RT-PCR, western blotting, and immunostaining revealed that NCSSs were composed of keratinocytes, melanocytes, and fibroblasts. Thus, tissue homogenization yielded skin cell suspensions that were similar to those in the ReCell system. Importantly, our method exhibited expression of appropriate growth factors (EGF and bFGF) to promote wound healing. β-Defensin2, which provides antimicrobial defense, was also present. However, further studies are needed to determine the origins of these factors as they may be secreted from living cells within the suspensions or could be a result of cell disruption by homogenization. Furthermore, application of NCSSs in a rat model of wound healing revealed that NCSSs improved epithelialization, skin adnexa regeneration, and collagen maturation at 1 week after wounding. Thus, these data suggested that application of the NCSS promoted improve wound healing, both in terms of rate and quality. Further studies with more animals are needed to confirm these results.

In addition to the above advantages of our system (i.e., lack of chemicals and rapid procedure), the entire process could be performed by the clinician and nurse in the operating room without additional help from laboratory personnel because of the simplicity of the tissue homogenization methods. Furthermore, it is expected that this procedure would only need approval of the hospital's Institutional Review Board (IRB) because the method does not require the use of medical devices, which generally need official prior approval from national authorities (i.e., the United States Food and Drug Administration). Lastly, the

procedure is also expected to be low-cost and effective, requiring only tube, beads, and strainer.

This NCSS method is expected to have applications in many different surgical fields. For example, we are in the process of obtaining IRB approval for the use of NCSS in patients with deep dermal burns. Small sections of skin harvested from the scalp area would not leave a scar, and the NCSS could be prepared from this tissue and applied to improve healing on the burn wound. In addition, NCSS application may decrease donor morbidity due to the small amount of tissue used. Almost all procedures for skin grafting leave remnant skin pieces because the electrical dermatome harvests skin in a rectangular shape, while the wound site for grafting is generally round. This remnant skin could be processed to prepare NCSSs to heal the donor site and could prevent donor pigmentation and scarring. Some clinicians have already used ReCell for pediatric donor sites.[15] Moreover, noncultured skin cell suspensions have been applied in dermatologic fields. NCSSs containing melanocytes have been shown to cause repigmentation of vitiligo lesions[16] and hypopigmented burn scars.[17] Thus, future studies should examine the use of NCSSs in fields ranging from burn management to cosmetics because our NCSSs also contained melanocytes. This study had a few limitations. First, NCSSs have not been studied in burn patients; thus, the effectiveness of this method in human burn wounds has not been confirmed. As stated above, we are currently working on obtaining IRB approval for such studies. Secondly, it is not clear whether the effects on wound healing are caused by keratinocytes or growth factors/cytokines within the NCSS. Finally, additional studies are still required to confirm the effects of other components within the NCSS besides cells because small molecules and other particles (less than 100 μm) were not removed from the NCSS before use.

V. CONCLUSION

Our study demonstrated that tissue homogenization could be used for preparation of NCSSs without the use of chemicals. This new method also isolated viable skin cells, yielding cell numbers and viabilities comparable to those of traditional methods. NCSSs containing viable skin cells and some growth factors promoted wound healing in an animal model. Thus, this method may be useful for improving wound healing in burn patients.

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ABSTRACT(IN KOREAN)

세포생존력과 상처치유력을 보존할 수 있는 비배양피부세포액을 만드는 새로운 방법에 관한 연구

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목적: 비배양피부세포액은 화상환자의 상처치료를 위해 사용되어져 왔다. 하지만 세포액을 만드는 과정에 시간이 많이 소요되고 화학물질이 사용에 따른 잠재적 유해성의 단점이 있다. 이에 저자들은 화학물질의 사용 없이 조직분쇄기를 사용하여 비배양피부세포액을 만들어 보고자 하였다.
방법: 세포수와 세포생존력은 화상환자의 조직에서 얻은 피부를 분쇄한 후 트리판블루를 사용하여 측정하였다. 세포의 형태는 전자현미경사진과 역전사중합효소연쇄반응을 이용하여 세포표지자를 분석하였다. 세포액내 세포형태를 구별하기 위해 면역형광염색을 시행하였다. 상처치유력은 ELISA법을 사용하여 성장인자들을 측정하였다. 비배양피부세포액의 임상사용 가능성을 확인하기 위해 누드랫 모델을 사용하여 분석하였다.
결과: 조직분쇄기를 적절히 셋팅한 후에 얻은 세포생존력은 52%에서 89%의 범위였다. 세포액에서 각질세포, 섬유아세포, 멜라닌세포가 확인되었고 주사전자현미경사진은 각질세포와 유사한 세포모양을 보여주었다. 세포액에는 표피성장인자, 섬유아세포성장인자등의 여러 성장인자가 존재하였다. 동물실험에서는 비배양피부세포액이 피부부속기의 재생과 상피형성을 촉진시켰다.
결론: 조직분쇄기를 이용하여 세포생존력과 상처치유력을 저해하지 않고 비배양피부세포액을 만들 수 있었다. 또한 이 과정은 빠르고 화학물질의 사용이 필요하지도 않았다.

핵심되는 말 : 각질세포, 비배양피부세포액, 화상